USSN: 09/616,283; Art Unit: 1645
Attorney Docket No. VRXB-P01-001
NOT FOR ENTRY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: GOODNOW	
Serial No: 09/616,283	Art Unit: 1645
Filed: July 14, 2000	Attorney Docket No. VRXB-P01-001
For: SYSTEM FOR DETECTING BACTERIA IN BLOOD, BLOOD PRODUCTS, AND FLUIDS OF TISSUES	Examiner: J. Hines

Assistant Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 35 U.S.C. §1.132

Sir:

I, Jeffrey A. Hall, Ph.D., of Franklin, MA, hereby declare as follows:

1. I am the Director of Assay Development at Verax Biomedical, Inc., the assignee of the present application. I have been conducting research in immunoassay development in the field of testing blood, blood products, and tissue for 13 years. Accordingly, my curriculum vitae is attached as Appendix A.

2. I have read the above-identified application, the pending claims, the Office Action mailed on February 11, 2003, and the Advisory Action mailed on June 19, 2003.

3. I understand that the Examiner has stated that the invention as described and claimed in the above-identified application is obvious in view of the teachings of Chan (EP 461,462), McLaughlin (U.S. Patent 4,683,196), Tadler et al. (*J. Clin. Lab. Anal.* 3: 21-25 (1989)), and Chang et al. (U.S. Patent 5,200,323).

4. The Examiner states that McLaughlin teaches antibodies which specifically bind to gram negative bacteria in order to determine their presence and/or absence while Tadler et al., teach well known binding agents that bind lipotechoic acid of gram-positive bacteria in assays. See Office Action dated February 11, 2003. Accordingly, the Examiner states that it would have been "prima facie obvious to modify the simultaneous multiple analyte detection immunoassay taught by Chan by incorporating a set of binding agents as taught by McLaughlin and Tadler et al."

5. I have reviewed the disclosures of both Tadler et al. and McLaughlin. For the reasons set forth below and the accompanying experimental data presented, I believe that the antibodies in these documents fail to demonstrate broad pan-generic cross-reactivity and detection at a level of sensitivity to be effective in detecting clinically relevant amounts of bacteria in a blood or blood products as required by the claims.

When?

6. Verax Biomedical, Inc., has developed pan-generic antibodies immunoreactive with the Gram-negative antigen lipopolysaccharide (LPS) and pan-generic antibodies immunoreactive with the Gram-positive antigen lipoteichoic acid (LTA). The pan-generic activity and sensitivity of these antibodies has been compared with the closest commercially available antibodies that are being marketed for pan-generic reactivity. We set forth below the comparative results obtained for both the Verax gram positive as well as the gram negative antibodies.

Gram-P sitive Antibodies

7. The Examiner states that Tadler et al disclose well known binding agents that bind the lipoteichoic acid (LTA) of the Gram-positive bacteria. We purchased commercially available antibodies that bind the LTA of the Gram-positive bacteria [Appendix B] and performed a side-by-side comparison with the Verax antibodies (VERAX PGD BA-3).

8. The specification as filed discloses how to make and use pan-generic gram positive antibodies. Example 9 of the application demonstrates to one of ordinary skill in the art that the Verax monoclonal antibody clone 96-110 (described in WO 98/57994 by Fisher et al.) shows pan-generic reactivity with the LTA of seven Gram-positive bacteria as depicted in Figure 5 of the application. [Appendix C]. The Verax antibodies show pan-generic activity against a range of bacteria that have been identified as contaminants in blood and blood products in three major national transfusion reaction studies including the BaCon Study in the United States, the Hemovigilance study in France, and the SHOT study in the United Kingdom.

9. In addition, we conducted further assays using Verax antibodies, such as VERAX PGD BA-3, in evaluating the ability of these antibodies to detect clinically relevant amounts of bacteria and be useful in constructing meaningful screening assays. These results are presented below:

When invented? Check dates

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PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM POSITIVE BACTERIA

Vendor	Antigen	TEST BACTERIA: Staph epidermidis	Staphylococcus aureus	Staph lugdunensis	Bacillus subtilis	Group B Streptococcus	Group G Streptococcus	Enterococcus faecalis	Corynebacterium sps	Clostridium perfringes
HyCult Biotech	G+ LTA	28.1	1.2	1.4	1.3	1.5	3.8	1.9	3.8	1.7
Biogenesis Inc.	G+ LTA	9.2	16.3	1.1	2.9	1.5	6.7	1.3	6.7	2.2
VERAX PGD BA-3	G+ LTA	62.6	7.6	12.5	20.3	5.6	14.8	5.8	14.8	4.7
VERAX PGD BA-4	G+ LTA	77.9	30.1	71.3	10.2	6.6	10.8	21.4	10.7	2.8

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS. A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

less sensitive → detect all but

As can be seen from the above data, the commercially available antibodies from HyCult Biotech and Biogenesis, Inc. were not truly pan-generic with respect to the detection of the LTA on Gram-positive bacterium. In contrast, the Verax antibodies detected 11 species of bacterium of Gram-positive bacterium routinely found in contaminated blood.

See results above and Figure 5.

10. As can be seen from the results above, to be effective in detecting clinically relevant amounts of bacteria a signal: noise ratio greater than 2 is required for constructing a meaningful assay. The Verax antibodies showed greater pan-generic reactivity in an immunoassay as compared to the commercially available antibodies.

11. We now provide a side-by-side comparison of the pan-generic reactivity of the Verax antibodies to the Tadler et al. antibodies. Tadler et al. discloses an immunoassay for the detection of the LTA on gram positive bacteria. A close review of their experimental data is limited to binding and detection of the *Streptococcus* species and *Staphylococcus* species. In total, Tadler et al. show detection of 5 species of gram-positive bacteria See Figure 2 of Tadler et al. In contrast, the Verax antibodies are capable of pan-generic binding and detection of at least 11 Gram-positive bacterial species.

12. We further provide a comparison of the sensitivity of the Verax antibodies to the Tadler et al. antibodies. Figure 2 of Tadler et al. shows that only 2 bacterial species, i.e., *Streptococcus mutans* and *Staphylococcus epidermidis*, are detected at clinically relevant amounts, $5x10^5$ CFU/50µl (i.e., $1x10^7$ CFU/ml). Thus, at this level of sensitivity the Tadler antibodies are not truly pan-generic. The bacteria *Staphylococcus aureus* is detected at $5x10^6$ CFU/50µl (i.e., $1x10^8$ CFU/ml), a level that is not clinically relevant. Additionally, the Tadler et al. immunoassay was unable to detect *Staphylococcus faecuim* at $5x10^6$ CFU/50µl ($1x10^8$ CFU/ml) suggesting that the antibodies are only able to cross-react with LTA on certain Staphylococcus *spp.* and Streptococcus *spp.*

13. In contrast, the following Table set forth both the pan-generic cross-reactivity of the Verax antibodies, as well as the sensitivity of these antibodies, demonstrating a greater degree of sensitivity in detecting clinically relevant amounts of bacteria in contaminated blood or blood products ($1x10^2$ CFU/ml – $1x10^6$ CFU/ml).

GRAM POSITIVE RAPID TEST SIGNAL (G/DENS)

CFU/ml	S. epidermidis	S. aureus	S. lugdunensis	B. cereus	GRP B Strep	GRP G Strep	S. pyogenes	E. faecalis	C. minutissimum	C. perfringes	CFU/ml
1.0 E5	17.26	1.04	13.21	12.24	0.26	16.07	nt	13.95	6.82	11.20	1.0 E5
5.0 E4	nt	nt	3.20	9.91	nt	nt	21.77	nt	nt	nt	5.0 E4
1.0 E4	8.81	0.41	0.36	2.45	0.04	1.43	10.32	3.19	2.03	8.67	1.0 E4
5.0 E3	3.98	nt	nt	nt	0.04	nt	nt	0.44	nt	nt	5.0 E3
1.0 E3	0.83	0.24	0.17	0.29	nt	0.46	0.51	0.27	1.26	2.91	1.0 E3

*BOXED CELL = MINIMALL DETECTABLE CONCENTRATION
**G/DENS = REFLECTANCE SIGNAL, ANY G/DENS > 0.25 IS VISIBLE
***nt = NOT TESTED

Gram-Negative Antibodies

14. The Examiner states that McLaughlin disclose antibodies which specifically bind to gram-negative bacteria. McLaughlin discloses mouse and rabbit antibodies that bind to the Lipid A core of the LPS on Gram-negative bacteria. We obtained commercially available mouse antibodies that are marketed as being anti-LPS core or anti-endotoxin [Appendix D] from HyCult Biotech, Virostat, QED, and Biogenesis, Inc and conducted a side-by-side comparison to the Verax antibodies.

15. The specification as filed discloses how to make and use pan-generic gram negative antibodies. Example 9 of the application demonstrates to one of ordinary skill in the art that the Verax monoclonal antibody clone 26-5 (commercially available from Biodesign International) shows pan-generic reactivity with the LPS of seven Gram-negative bacteria as depicted in Figure 6 of the application. [Appendix E].

16. McLaughlin antibodies are reactive with gram-negative bacteria such as *Neisseria, Chlamydia, and Salmonella*. In contrast, the Verax antibodies show pan-generic activity

against a range of bacteria that have been identified as contaminants in blood and blood products in three major national transfusion reaction studies including the BaCon Study in the United States, the Hemovigilance study in France, and the SHOT study in the United Kingdom. These contaminants include both pathogenic as well as non-pathogenic bacterial species such as *Yersinia entercolitica* and *Proteous miribilis*, along with other common soil-borne bugs. The McLaughlin antibodies appear to be effective mainly against the pathogenic gram negative bacterial species (e.g., *Neisseria* and *Chlamydia*).

17. In addition, we conducted further assays using Verax antibodies, in evaluating the ability of these antibodies to detect clinically relevant amounts of bacteria and be useful in constructing meaningful screening assays. These results are presented below:

PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM NEGATIVE BACTERIA

		TEST BACTERIA										
Vendor	Antigen	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Acinetobacter baumanii*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella enteritidis*	*Yersinia entercolitica*	*Proteus mirabilis*	*Serratia marcescens*
HyCult Biotech	LPS	1	1	1.1	1.2	1.1	1.2	1	1	1	1.3	1.3
Virostat	LPS	1	1	1	1	1	1	1	1	1	1	1.1
QED	LPS	1	1	1	1	1	1	NT	NT	NT	NT	NT
VERAX PGD BA-1	LPS	12.1	11.1	12.5	12.8	12.3	9.6	9.7	11.2	11.4	10.6	12.3
VERAX PGD BA-2	LPS	11.2	16.5	14.2	9.5	7.8	13.5	9.2	8.5	12.1	19.5	22.7

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS
A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

18. As can be seen from the results above, a signal: noise ratio greater than 2 is required for constructing a meaningful assay. The Verax antibodies showed greater effectiveness and

could be detected in an immunoassay as compared to the commercially available antibodies.

19. In addition, the following Table sets forth the sensitivity of the Verax gram negative antibodies, demonstrating a greater degree of sensitivity in detecting clinically relevant amounts of bacteria in contaminated blood or blood products ($1x10^2$ CFU/ml – $1x10^6$ CFU/ml).

VERAX PLATELET PGD ASSAY: ANALYTICAL SENSITIVITY

	GRAM NEGATIVE RAPID TEST SIGNAL (G/DENS)										
CFU/ml	*E. coli*	*Ps. aeruginosa*	*E. aerogenes*	*K. oxytoca*	*E. cloacae*	*A. baumanii*	*K. pneumoniae*	*S. enteritidis*	*P. mirabilis*	*S. marcascens*	CFU/ml
1.0 E5	4.51	2.16	9.13	8.66	1.01	7.71	5.30	1.14	1.38	0.83	1.0 E5
5.0 E4	4.10	1.45	7.70	7.08	0.77	7.51	4.40	0.70	0.88	0.71	5.0 E4
1.0 E4	2.75	0.79	6.88	3.31	0.82	4.79	2.34	0.50	0.66	0.69	1.0 E4
5.0 E3	1.34	0.67	4.81	1.46	0.73	2.98	1.21	0.48	0.24	0.16	5.0 E3
1.0 E3	0.99	0.32	4.10	0.25	0.75	1.48	0.57	0.04	0.09	0.01	1.0 E3

20. In addition, to the antibodies described in the application, Verax has developed additional gram positive and gram negative antibodies using the methods of making and/or selecting antibodies as set forth in the application. Antibodies having potential use for broadly detecting Gram-positive bacteria and Gram-negative bacteria are described at lines 16-30 of page 25 and lines 1-2 of page 26 of the application. The process by which antibodies are evaluated and selected are described in the application at lines 1-22 of the application. Selection of other types of binding agents to be used in the present invention are disclosed at lines 23-31 of 26 and lines 1-6 of page 27.. The Verax antibody PGD BA-3

is the same antibody as described in Example 9 of the specification. The Verax antibody PGD BA-4 as set forth in the Gram-positive comparison Table was developed using the methods described in the specification and are pan-generic for the LTA. Further, the Verax antibodies PGD BA-1 and PGD BA-2 as set forth in the Gram-negative comparison Table were developed using the methods described in the specification and are pan-generic for the LPS.

21. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Jeffrey A. Hall

Dated: Signature:

ROPES & GRAY LLP
ONE INTERNATIONAL PLACE BOSTON, MA 02110-2624 617-951-7000 F 617-951-7050
BOSTON NEW YORK SAN FRANCISCO WASHINGTON, DC

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